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Isolation, Screening, and Characterization of Cellulolytic Bacteria from Domestic Wastewater in Mumbai

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ABSTRACT

The increasing accumulation of cellulosic waste in urban environments necessitates efficient and sustainable degradation strategies, particularly in wastewater treatment systems. This study aimed to isolate, screen, and characterize cellulolytic bacteria from domestic wastewater samples collected from the Mithi River at the Taximan Colony area in Kurla, Mumbai—an urban site heavily polluted with domestic effluents. Enrichment was performed using Bushnell and Haas broth with filter paper as the sole carbon source, followed by isolation on carboxymethyl cellulose (CMC) agar plates. Primary screening based on Congo red staining revealed several isolates with significant cellulolytic efficiency. Secondary screening using the DNSA method quantified cellulase activity, with isolate CMR01 showing the highest enzyme production. The enzyme exhibited stability over a broad range of pH (5–12), temperature (20–50°C), and moderate salinity, indicating its suitability for application in diverse environmental conditions. Biochemical tests and 16S rRNA gene sequencing identified the potent isolate as Enterobacter cloacae strain MR12, with the sequence submitted to NCBI (Accession No. PQ849158). The findings highlight the potential of wastewater-derived cellulolytic bacteria, particularly Enterobacter cloacae, as effective agents for biodegradation of cellulosic material, offering promising applications in environmental biotechnology and sustainable wastewater management.

Keywords: Cellulolytic bacteria, Enterobacter cloacae, Mithi River, Wastewater microbiology, Cellulase enzyme, Bioremediation, Environmental biotechnology, CMC agar, 16S rRNA gene sequencing, Domestic sewage, Cellulosic waste degradation.

INTRODUCTION

Cellulose is the most abundant organic polymer on Earth, constituting a major component of plant biomass and agricultural waste. Its effective biodegradation is essential for recycling carbon in nature and for sustainable management of organic waste (Lynd et al., 2002). Cellulolytic bacteria play a crucial role in this process by producing cellulase enzymes that hydrolyze cellulose into simpler sugars (Bhat et al., 1997). These microorganisms have gained considerable attention for their potential applications in various industrial sectors, including biofuel production, agriculture, paper and pulp industries, and, notably, in wastewater treatment for the bioremediation of organic pollutants (Kuhad et al., 2011).

Urban water bodies, especially those contaminated with domestic sewage and organic waste, provide a rich microbial ecosystem where naturally occurring bacteria adapt to decompose complex organic matter (Gupta et al.,2020). The Mithi River in Mumbai, heavily impacted by urban and domestic effluents, represents one such environment where microbial diversity thrives under polluted conditions. Exploring such sites for cellulolytic bacteria offers a dual advantage: discovering novel enzyme-producing strains and understanding their adaptation to environmental stress, which is critical for practical biotechnological applications (Sartale et al., 2012).

This study focuses on the isolation, screening, and characterization of cellulolytic bacteria from domestic wastewater samples collected from the Mithi River. The aim is to identify potent bacterial strains capable of producing stable cellulase enzymes under varying physical and chemical conditions. The research further seeks to characterize these isolates biochemically and molecularly to assess their suitability for future use in the bioconversion of cellulosic waste and wastewater treatment processes. This investigation not only contributes to the field of environmental microbiology but also supports the development of eco-friendly solutions for managing urban organic waste.

MATERIALS AND METHODS

2.1 Sample collection:
Water and sludge samples were collected from the Mithi River at the Taximan Colony area in Kurla, Mumbai, to isolate cellulolytic bacteria. This site was selected due to its high level of contamination from domestic wastewater, providing a rich microbial environment influenced by urban pollution. The sampling aimed to explore bacterial diversity and

potential cellulase activity in response to such conditions. An integrated sampling method was employed, and all samples were transported at 4°C to ensure preservation for immediate laboratory processing (Singare et al., 2011).

2.2. Enrichment and isolation of cellulolytic bacteria:

The water samples were enriched in Bushnell and Haas broth minimal medium, with filter paper as the carbon source. The medium composition included 1 g/L Whatman filter paper No. 1, 0.2 g/L Magnesium sulfate, 0.02 g/L Calcium chloride, 1 g/L Monopotassium phosphate, 1 g/L Dipotassium phosphate, 1 g/L Ammonium nitrate, 0.05 g/L Ferric chloride, and a pH of 7.0 ± 0.2. Enrichment was carried out at 28 ± 2°C for 120 hours with shaking at 120 rpm. Following enrichment, cellulolytic bacteria were isolated on carboxymethyl cellulose (CMC) agar plates using the spread plate technique.

The CMC agar medium composition included 2 g/L Carboxymethyl cellulose, 1.84 g/L Na_2HPO_4, 11.8 g/L KH_2PO_4, 1.4 g/L $(NH_4)_2SO_4$, 0.1 g/L Magnesium sulfate, and 17 g/L Agar. The plates were incubated at 28 ± 2°C for 48 hours, and colonies with morphological variations were selected and transferred into nutrient broth for further analysis.

To obtain pure cultures, bacterial isolates grown in nutrient broth were streaked onto nutrient agar plates and incubated at 28 ± 2°C for 24 hours. This process was repeated twice. The purified isolates were stored as glycerol stocks (25% v/v) at -20°C to preserve their viability for subsequent experiments and analysis (Zerva et al., 2019).

2.3 Screening for Cellulolytic Activity

The purified bacterial isolates were screened for extracellular cellulase production using CMC agar plates. After streaking and 48 hours of incubation, plates were stained with 0.2% Congo red for 30 minutes and washed with 1M NaCl to visualize clear zones indicating cellulose hydrolysis (Gohel et al., 2014; Porkavi et al., 2021). The diameters of the hydrolysis zones and colonies were measured in millimeters, and cellulolytic efficiency was calculated using a standard formula.

$$\text{Cellulolyticefficiency} = \frac{\text{Diameterofclearzone} - \text{Colonydiameter}}{\text{Colonydiameter}} \times 100$$

2.4 Secondary screening for cellulase production:

Bacterial isolates showing high cellulolytic efficiency were selected for enzyme activity analysis using the DNSA method (Malik et al., 2021). Isolates were cultured in enzyme production medium containing 5g/LCarboxymethyl cellulose, 0.5 g/L$MgSO_4$,1 g/L$(NH_4)_2SO_4$,1 g/LKH_2PO_4, 0.5 g/L yeast extract, 1g/L glucose, pH 7 at 37°C for 24 hours with shaking (120 rpm). After centrifugation at 5000 rpm for 10 minutes, the supernatant was used as crude enzyme. Cellulase activity was measured by incubating 0.5 ml of crude enzyme with 0.5 ml of 2% CMC (in 0.05M sodium citrate buffer, pH 4.8) at 50°C for 30 minutes. DNSA reagent (3 ml) was added, followed by boiling for 5 minutes and cooling. Absorbance was read at 540 nm, and enzyme activity was calculated using a standard glucose curve (Ghose et al., 1987).

2.5 Effect of physical parameters on cellulase activity

The stability of cellulase enzyme over wide range of pH (pH 5 to pH 9), temperature (20°C to 80°C) and salt concentration (0-5%) was checked to obtain potent candidates with stable enzyme activity at various environmental conditions (Bakare et al., 2005).

2.6 Identification of Bacterial Isolates:

Biochemical characterization of selected bacterial isolate was conducted based on colony morphology, Gram staining, and standard biochemical tests, including IMViC, urease, motility, catalase, and sugar utilization, using overnight cultures. Results were validated with positive and negative controls as per Bergey's Manual of Determinative Bacteriology (Bergey's Manual of Determinative Bacteriology).

Molecular identification was carried out through 16S rRNA gene sequencing. Genomic DNA was extracted via heat treatment, and the 16S rRNA gene was amplified using universal primers UNI1500F and UNI1500R. PCR product quality was assessed by gel electrophoresis and Nanodrop, and sequencing was done by Blot Solutions, India. The resulting sequences were analyzed using BLAST, and phylogenetic analysis was performed using CLUSTALW and the neighbor-joining method (Sevak et al., 2023).

2.7 Statistical Analysis

The qualitative and quantitative estimation of cellulase production from each bacterial isolate was performed in triplicates. The mean and standard deviation measurement was employed to describe central tendency and variability of data, respectively. The mean represents the average value of the three readings, providing a central reference point, while standard deviation quantifies the extent to which individual readings deviate from mean.

3. Results and discussion

Cellulolytic bacteria were isolated from domestic wastewater sample collected the Mithi River at the Taximan Colony area in Kurla, Mumbai using cellulose-containing media, followed by culturing and biochemical characterization. Potent strains with high enzyme activity and environmental stability were screened.

3.1 Qualitative Estimation of Cellulase Production

To qualitatively assess cellulase production, bacterial isolates grown on CMC agar plates were evaluated based on their ability to hydrolyze CMC, resulting in

clear zones that indicate enzymatic activity. Congo Red staining helped differentiate hydrolyzed and non-

Table 1: Qualitative and Quantitative Estimation of Cellulase Enzyme

Sr. No.	Isolate	Cellulolytic efficiency	Enzyme activity (IU/ml)
1	CM1	442.67 ± 22.90	0.788 ± 0.050
2	CM2	611.67 ± 10.21	0.742 ± 0.016
3	CM3	531.33 ± 16.92	-
4	CM4	414.33 ± 12.90	-
5	CM5	639.00 ± 47.57	-
6	CMR01	828.67 ± 3.79	0.868 ± 0.041
7	CMR02	510.67 ± 10.07	-
8	CMR03	450.67 ± 20.01	0.516 ± 0.022
9	CMR04	616.00 ± 14.42	0.716 ± 0.020
10	CMR05	326.33 ± 30.02	-
11	CMR06	841.00 ± 50.21	0.742 ± 0.025
12	CMR07	434.00 ± 17.78	0.525 ± 0.058
13	CW01	355.00 ± 26.06	0.731 ± 0.010
14	CW02	553.67 ± 46.69	0.555 ± 0.019
15	CW03	529.00 ± 42.67	0.726 ± 0.003
16	CW04	556.00 ± 27.18	0.622 ± 0.052

hydrolyzed areas, with zone formation indicating cellulase activity. Few promising isolates were shortlisted in the primary screeningshowing the zone of clearance on CMC agar plates stained with 0.1% congo red dye (Figure 1).

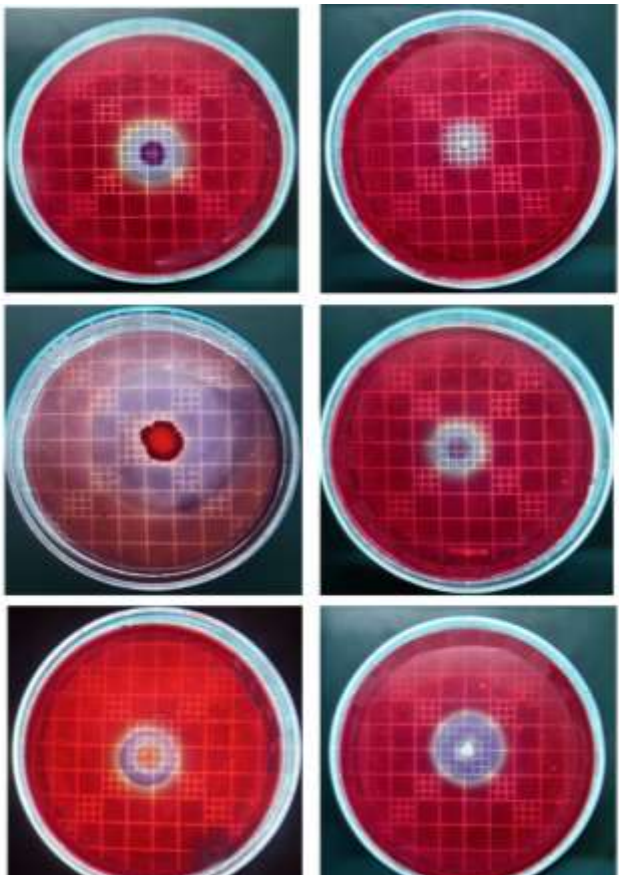

Figure 1: Zone of clearance due to cellulase activity of bacterial isolates on CMC agar plates stained with 0.2% congo red

3.2 Determination of Cellulase Activity

To quantify cellulase production, selected isolates from the primary screening were cultivated in a production medium, and enzyme activity was measured using the DNSA spectrophotometric method. Few isolates showing both high enzyme activity and stability under varying physical conditions were selected as potent candidates for cellulase production(Table1). Remarkably, this is the first known study to isolate cellulolytic bacteria from sewage, expanding the scope of environmental microbiology. The enzyme activity observed aligns with previous studies, such as that by Niranjan et al. (2013), who reported 0.0423 U/mL from municipal waste-derived isolates. These findings underscore the potential of the selected isolates in bioremediation of cellulosic waste in wastewater treatment systems.

3.3 Effect of Physical Parameters on Cellulase Production:

The effect of physical parameters like pH, temperature, and salinity on cellulase production was studied selected isolates. Isolates, especially CMR01, showed

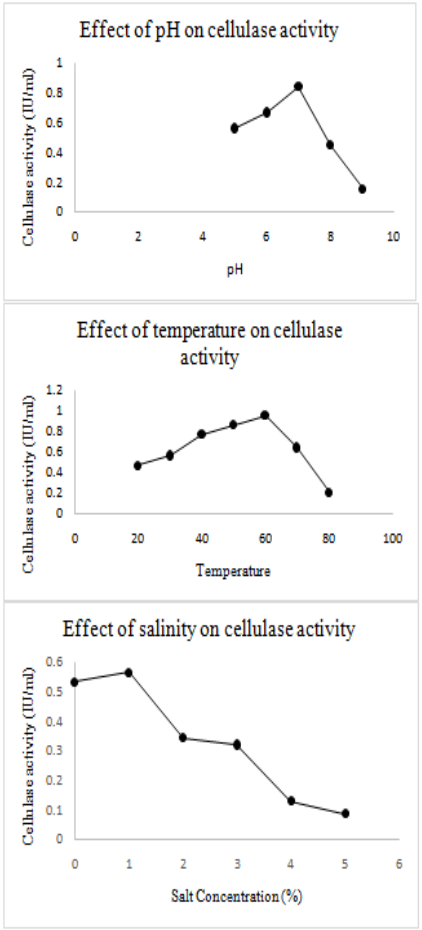

Figure 2: Effect of physical parameters on cellulase activity of isolate CMR01

stable enzyme activity across pH 5–12 and retained over 60% activity between 20–50°C. Enzyme activity declined at high salinity, yet the selected isolate maintained notable salt tolerance (Figure 2). These traits highlight their potential for bioremediation in fluctuating environmental conditions, including saline and coastal wastewater systems.

3.4 Biochemical Characterization and Identification of Bacteria:

The selected bacterial isolate CMR01 was characterized based on its colony morphological characteristics and biochemical behaviour. Bacterial isolate was found to be Gram negative cocci which produced rod shaped, irregular, creamish opaque colonies on a nutrient agar plate and was motile in nature. It could metabolize citrate as the only carbon source and also produce catalase enzyme which indicates that it is capable of neutralizing oxide radicles and overcoming oxidative stress. Also, it could metabolize carbohydrates such as glucose, maltose, mannitol, sucrose and xylose with the production of acid and gas.

The sequence of amplified 16 S rDNA of bacterial isolate CMR01 was analysed by sequence homology search through BLASTn available at NCBI. The CMR01 isolate was identified as Enterobacter cloacae strain MR12 belonging to the Enterobacteriacefamily. Biochemical characteristics of the isolated bacteria also supported the identity of the isolate as Enterobacter Sp. thus further confirming the identity of bacteria.16S rDNA sequence of the isolate was submitted to NCBI with accession no PQ849158.

CONCLUSION

The present study successfully isolated, screened, and characterized potent cellulolytic bacteria from domestic wastewater samples collected from the Mithi River at the Taximan Colony area in Kurla, Mumbai. Utilizing enrichment and selective culturing techniques, several bacterial isolates exhibiting significant cellulolytic activity were obtained. Among them, the isolate CMR01 demonstrated high cellulolytic efficiency, notable enzyme activity, and remarkable stability across varying environmental conditions, including a wide range of pH, temperature, and salinity.

Biochemical and molecular identification confirmed CMR01 as Enterobacter cloacae strain MR12. This strain's ability to produce stable cellulase enzymes under diverse environmental stresses indicates its strong potential for application in bioremediation of cellulosic waste and enhancement of wastewater treatment processes. Notably, this is the first known report of cellulolytic Enterobacter cloacae being isolated from domestic sewage, broadening the understanding of microbial diversity in polluted aquatic ecosystems and offering promising candidates for sustainable biotechnological interventions.

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